digestive tract tumors in the 23 February 1996 issue of *Cell*. In the 5 April 1996 issue of the same journal, the team detailed defects—mostly deletions of genetic material—at the fragile site in cultured cells from small-cell and nonsmall-cell lung tumors. "We want to determine if people differ in fragility . . . the molecular basis of fragility, and whether people are predisposed to cancer depending on the fragility of their *FHIT* gene, but we don't have the answer yet," Croce says.

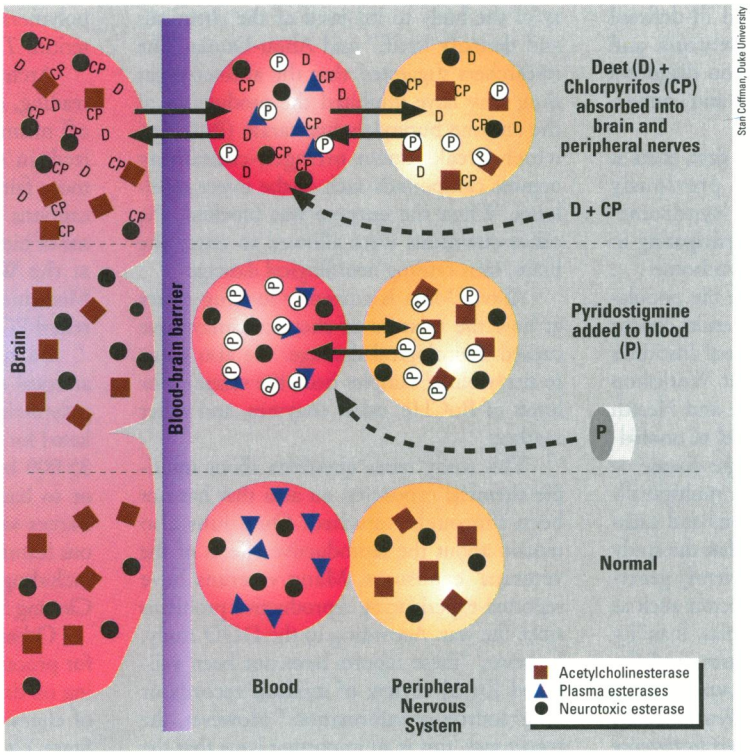
George T. O'Connor, an expert on secondhand smoke at the Boston University School of Medicine, has reservations about studies showing defective genes in cancer cells, however. "The problem with studies of this nature is that when cells undergo malignant transformation, many aspects of those cells become messed up. So the fact that you look at the actual tumor cells and you see that there are all these messed up genes doesn't mean that in any way [the gene] was causal. It could be a result of the fact that cancer has developed that the genes are messed up." This prompts the question of whether fragile sites are more fragile in some individuals than in others.

Croce says research indicates that "in patients with cancer predisposition, their fragile site is more fragile than in normal individuals. That raises the issue of whether we all differ in fragility and whether some specific environmental agents can affect those fragile sites. . . . We believe that because of its intrinsic fragility, *FHIT* might be hit by a variety of environmental agents: biological (like viruses), physical, or chemical (like benzopyrene in cigarette smoke) . . . causing a mutation." Environmental damage to the gene could provide the first of several genetic hits required to cause a cell to become cancerous.

"We are convinced that the changes in *FHIT* occur very early [in the multistep path to malignancy]," Croce said. "So if you could stop the process when it's just started, you might prevent the development of lung cancer. If we can detect those changes early on, we can probably devise therapies just to stop the growth of the cells or to kill the cells that have *FHIT* alteration."

Croce cites a huge subpopulation that could benefit from knowing whether carcinogens in cigarette smoke have already attacked their *FHIT* gene: the 42 million ex-smokers in the United States. Smokers, ex-smokers, and nonsmokers affected by secondhand smoke may all benefit from the discovery of *FHIT*, he says.

Furthermore, secondhand smoke may affect many more people than formerly thought, according to a 24 April 1996 report in *JAMA*. The data, collected between 1988 and 1991, show that nearly 90% of nonsmokers four years old and older had detectable levels of cotinine, a major metabolite of nicotine, in their blood. Cotinine levels indicate recent exposure to nicotine. "Even people who say 'No, there's no one in my home who smokes,' you still may find they have measurable cotinine," says O'Connor. "In the typical day-to-day life of an American, we come into contact with cigarette smoke."



Stan Coffman, Duke University

Mixed results. Recent research on the effects of exposure to mixtures of pyridostigmine, which blocks enzymes, and DEET and permethrin, which are able to penetrate the brain, may offer clues to the symptoms of Persian Gulf War veterans.

New Persian Gulf Possibilities

The possibility of a "Persian Gulf syndrome" continues to perplex researchers and veterans. Since the Persian Gulf War, about 30,000 of approximately 697,000 veterans have complained of symptoms such as joint pain, headaches, fatigue, memory loss, depression, sleep disturbance, and diarrhea. Some researchers and veterans have suggested that a syndrome caused by environmental exposures during the war is affecting Persian Gulf veterans, and researchers have been looking for explanations.

The Department of Defense (DOD) recently released results from a study that evaluated 18,598 Persian Gulf War veterans. The study found no evidence of a syndrome, and diagnosed over a third of the veterans as suffering from psychological symptoms and about a tenth of the soldiers as healthy. Meanwhile, several university researchers collaborating on another study have suggested that the symptoms could be linked to a combination of chemicals that was administered to soldiers to protect their health during the war.

The DOD and the Depart-ment of Veterans Affairs developed a clinical program to evaluate veterans and identify exposures to occupational, environmental, chemical, and physical agents, as well as to vaccines and medications. The report, entitled *Comprehensive Clinical Evaluation Program for Persian Gulf War Veterans*, found that the veterans' symptoms are "routinely reported and not unique to the veterans."

According to the report, the most common self-reported exposures include passive cigarette smoke, diesel and other fuels, pyridostigmine bromide tablets used as an anti-nerve gas agent, oil smoke, tent and heater fumes, and personal pesticide use. The participating veterans were given primary diagnoses, and 80% were also given additional diagnoses. The most prevalent primary diagnoses were psychological conditions (18.4%); musculoskeletal and connective tissue diseases (18.3%); symptoms, signs, and ill-defined conditions (17.9%); respiratory diseases (6.8%); and digestive system diseases (6.3%).

When secondary diagnoses were also taken into account, the distribution was similar: musculoskeletal and connective tissue diseases (47.2%); symptoms, signs, and ill-defined conditions (43.1%); psychological conditions (36%); digestive system diseases (20.4%); skin and subcutaneous diseases (19.9%); nervous system diseases (17.8%); and respiratory diseases (17.5%).

Among the psychological diagnoses, the most common symptoms were tension headaches, depression, anxiety disorders, and adjustment reactions. Malaise and fatigue, sleep disturbance, and headache were the most frequent symptoms among the diag-

noses of symptoms, signs, and ill-defined conditions. Joint pain, osteoarthrosis, and backache were the most common diagnoses among those of musculoskeletal and connective tissue diseases.

According to the report, "to date, there is not clinical evidence for a previously unknown, serious illness or 'syndrome' among Persian Gulf veterans participating in the study. A unique illness or syndrome . . . would probably be detectable in the population of 18,598 patients." These results concur with the conclusions of a National Institutes of Health Technical Assessment Workshop on the Persian Gulf Experience and Health held in April 1994, where a panel of nonfederal experts found that "no single disease or syndrome is apparent, but rather multiple illnesses with overlapping symptoms and causes." The report also points out that the study was limited and cannot be sufficiently generalized to other veterans due to factors such as self-selection of patients, recall bias, inability to validate self-reported exposures, and the lack of an appropriate control group.

A recent study, entitled *Neurotoxicity Resulting from Coexposure to Pyridostigmine Bromide, DEET, and Permethrin: Implications of Gulf War Chemical Exposures*, has linked the combination of these chemicals to neurological damage. The study, led by Mohamed Abou-Donia, a professor of pharmacology at Duke University, was published in the May issue of the *Journal of Toxicology and Environmental Health*, and may have implications for research on a Gulf War syndrome.

Abou-Donia's team researched the neurological effects of the antinerve agent pyridostigmine bromide, the insect repellent DEET, and the insecticide permethrin on hens, which are recommended by the EPA for neurological testing because their nervous system resembles that of humans. The study found that, when administered individually, the compounds result in minimal toxicity, but when combined, they produce adverse neurological effects.

The hens were given doses of the chemicals that were three times the doses received by Persian Gulf soldiers. A group of hens given the chemicals individually suffered from minor effects. However, other groups of hens given two of the chemicals together developed diarrhea, weakness, shortness of breath, and inability to fly correctly. According to Abou-Donia, some of these symptoms were similar to those experienced by the veterans. Some of the hens in groups that were exposed simultaneously to all three chemicals became paralyzed or died.

Researchers hypothesize that the combination of chemicals results in a failure of the body's ability to neutralize them. "The mixture of chemicals seems to decrease the ability of the body to rid itself of the chemicals and detoxify itself," said Abou-Donia. The researchers suggested that the antinerve-gas drug may have inhibited the production of the enzyme butyrylcholinestarase (BuCHe), which breaks down nitrogen-containing organic compounds such as the insect repellents. When the enzyme was blocked, the other chemicals were allowed to enter the brain, thus causing neurological damage.

Abou-Donia is currently looking closer at how the mixture of chemicals may have caused neurological damage. He also plans to study blood samples from the veterans for levels of BuCHe, other enzymes, and other markers.

This study raises questions about multiple chemical exposures, an area that has not been thoroughly studied. Questions also remain about the reproductive effects of the veterans' exposures. Many veterans have reported experiencing reproductive problems since the war. According to the DOD study, however, "these reports have not been validated [by a] review of medical records or other sources of information." However, the report says, this is an important issue that the DOD will study further.

Deep Sea Microbes and DNA Cloning

Scientists are using microbes from ancient environments to enhance modern biotechnology. DNA polymerases from archaea, microorganisms that thrive in hydrothermal vents, make longer, more accurate DNA copies and work at higher temperatures in the polymerase chain reaction (PCR) than a commonly used bacterial enzyme.

Some archaea survive at above 100°C in the high-pressure, deep-sea vents that represent Earth's earliest environments, says John Baross, professor of oceanography at the University of Washington in Seattle. Baross collects archaea from the Pacific Ocean at depths of 2,000 meters or more, where seafloor spreading causes magma-heated fluids to spew forth. He has cultured about 300 isolates of *Thermococcus* and other hyperthermophilic archaea, which grow best at temperatures of 85–113°C.

Baross is seeking new enzymes to improve the efficiency of research and industrial reactions. PCR, for example, employs cycles of heat and cooling to denature and polymerize DNA. "What we're looking for in a new DNA polymerase is something that can amplify templates to greater than 10,000 bases, that has a very high fidelity with a variety of primers, and is more thermally stable," Baross says. Enzymes from high-temperature archaea have the advantage of withstanding cycling longer than the commonly used *Taq* polymerase, which is derived from the bacterium *Thermus aquaticus*.

Archaeal polymerases also have proofreading, or exonuclease, activity. They chew off mismatched bases as DNA is polymerized, in some cases making copies 10-fold more faithful to the original than *Taq*. But, cautions Wayne Barnes, an associate professor of biochemistry and molecular biophysics at the Washington University School of Medicine in St. Louis, the same function can retard PCR by degrading DNA primers.

Barnes found that mixing *Taq* and archaeal enzymes balanced proofreading and polymerization. The blend, now being marketed for "long PCR," makes DNA strands of 35,000 bases or more, rather than the 3,000 or so bases typically yielded by *Taq* alone, Barnes says. Archaeal polymerases with various features are now being sold by companies including New England Biolabs, Stratagene Cloning Systems, and Boehringer Mannheim.

Other archaeal enzymes are being tested for processing food and improving flow during oil drilling, says Robert Kelly, a professor of chemical engineering at North Carolina State University. But there may be drawbacks to using them because industrial applications can require tons of enzymes, which archaea don't produce readily in culture. The microbes are anaerobic, often grow slowly, and have unusual nutrient requirements; in addition, their enzymes are difficult to purify, Kelly says.

While archaea resemble bacteria, they're more closely related to eukaryotes, Baross writes in a new book, *Advances in Protein Chemistry* (in press). "With the link between high-temperature archaea and higher organisms," he says, "we may be able to ask questions like 'What is the origin of viruses?,' or 'What is the origin of certain genes?' "

Archaea may also offer clues to finding

James F. Holden

Promising primitives. A strain of deep sea microbes called *Thermococcus* are improving DNA cloning.